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ORIGINAL ARTICLE

Seedlings from marginal and core populations of European beech (*Fagus sylvatica* L.) respond differently to imposed drought and shade

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Abstract

Key message **Local adaptation largely segregates between traits affecting light responses and water relations, but population-level differences in stomatal conductance when growth is unconstrained converge among populations under water stress.**

Abstract Warming temperatures and altered precipitation patterns threaten plant populations worldwide. European beech (*Fagus sylvatica*) is a species that expresses both high phenotypic plasticity and local adaptation among populations. Beech seedlings' susceptibility to prolonged drought may be dictated by their immediate light environment. We tested whether seedlings of four beech provenances, from contrasting edaphoclimatic environments, expressed differences in trait responses to imposed water stress under sun and shade treatments. Populations from the southern range margin were expected to display greater water-stress tolerance and core populations' faster growth rates in the absence of abiotic limitations. Both high light and water stress induced differences in trait responses among provenances, but traits that failed to respond to our experimental treatments likewise did not segregate at the provenance level. Hence, those traits responding to light, e.g., increasing leaf flavonol index and leaf mass area, also tended to differ among provenances. Similarly, there was evidence of local adaptation among provenances in traits, like midday leaf water potential, responding to water stress. Exceptionally, there was a three way interaction water-×-light-×-provenance for stomatal conductance which converged among provenances under water stress. Leaf chlorophyll content also varied both with light and water in a provenance-specific manner. We found core provenances' growth traits to outperform others under favourable conditions, whereas southern and high-elevation populations displayed traits adapted to tolerate high irradiance. Only stomatal conductance produced a complementary interactive response between light-×-water across provenances, whereas other traits responded less to combined water stress and high irradiance than to either treatment alone.

Keywords Local adaptation · Phenotypic plasticity · Photoprotection · Climate change · Provenance trial · Drought stress

Introduction

Climate change across Europe is expected to drive the northwards range shift of tree species, due largely to warmer and drier conditions, facilitating better growth of populations at

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the leading range-edge but increasing the likelihood of mortality at the trailing range-edge (Pulido et al. 2019; Archambeau et al. 2020; Fréjaville et al. 2020). The consequences for species like *Fagus sylvatica* L. (henceforth beech) are of particular interest, as beech is an ecologically, culturally and commercially important species with a wide geographical distribution across Europe. To implement actions to mitigate effects of climate change, we should strive to understand processes underpinning plant fitness, including the relative importance of phenotypic plasticity and local adaptation to the sorts of environmental stresses that species are predicted to experience (Valladares et al. 2014). As seedlings are typically less able to resist stress than adult trees, the most important environmental filters act during the recruitment of tree seedlings into a population (Fraaije et al. 2015). Thus, studies on seedlings can provide relevant information on the eco-evolutive characteristics of populations in an adaptive context.

Tree seedlings often establish in an environment, where they are exposed to multiple chronic stresses. Although shade-tolerance in beech at the species level confers cross-tolerance, allowing trees to survive better under some environmental stresses (Valladares et al. 2007), this does not always apply to shade and drought which can interact to produce an antagonistic effect on the morphological and physiological acclimation responses of tree seedlings (Niinemets 2010). Potential antagonism in these functional-trait responses to combined water-and-light limitation can impede the recruitment of beech seedlings (Aranda et al. 2005; Robson et al. 2009). Two scenarios for the action of seasonal drought in forest understoreys are: (1) that in sunny canopy gaps, high evaporative demand and irradiance favour high photosynthetic rates but accompanied by water loss and photoinhibition, and (2) that in shaded understorey environments, carbon starvation can occur when stomata remain closed to maintain plant water balance under severe drought stress (Aranda et al. 2001), and respiratory losses exceed carbon gained through photosynthesis (Valladares and Niinemets 2008; Rodriguez-Calcerrada et al. 2010; Arend et al. 2016). Nevertheless, in arid environments, shade can sometimes benefit woody saplings (e.g., *Pistacia* and *Quercus*) by reducing leaf temperatures and photoinhibition, and promoting shade phenotypes that are typically better adapted to survive drought than sun phenotypes, despite the larger initial decreases in photochemical efficiency they suffer under equivalent drought stress (Valladares et al. 2005).

As a late-successional forest species, beech is shade-tolerant and relatively slow growing. It maintains a seedling bank in deep understorey shade, exploiting gaps or lighter shade providing favourable conditions for promotion to the canopy (Collet et al. 2002; Annighöfer et al. 2017). These increases in sunlight reaching the understorey provide the irradiance required to increase photosynthetic carbon gain, and physiological and morphological acclimation, as well as biochemical photoprotection, increasing cross-tolerance to environmental stressors (Tognetti et al. 1998; Scartazza et al. 2016). The relatively conservative functional strategy and shallow-rooting growth habit of beech means that the species is often considered vulnerable to changes in climate, in particular increased summer droughts (Jump et al. 2006; Geßler et al. 2006; Piovesan et al. 2008). Earlier research has shown that despite its shade tolerance, beech seedling mortality can be higher in understorey shade than sun-patches under drought conditions (Valladares and Pearcy 2002; Robson et al. 2009). This is thought to be either because of (1) greater soil drought in understorey shade due to the proximity of large trees' roots, (2) lower relative allocation to roots in the shade than in the sun, (3) the inability to utilise understorey sunflecks for carbon fixation due to restrictions on stomatal opening under drought, or (4) a functional trade-off limiting the capacity of phenotypes acclimated to shade to deploy drought avoidance mechanisms; reducing hydraulic conductivity through morphological acclimation and by increasing intrinsic water use efficiency (Aranda et al. 2001; Robson et al. 2009; Bartlett et al. 2012).

European beech has an interesting biogeographical history as it was eliminated from much of Europe during the last glaciation, apart from sheltered refugia around the Mediterranean and in the Balkans (Magri 2008; Tegel et al. 2014), from where it subsequently recolonised during the Holocene spreading from the south east of Europe (Giesecke et al. 2006). This has led to a highly structured genetic diversity across the current range, which stretches from central Spain in the south-west of Europe to Sweden in the north, and Poland and Ukraine in the east (Paule et al. 1984). This diversity is evident from neutral and adaptive molecular markers (e.g., Carsjens et al. 2014; Gömöry et al. 2015; Bontemps et al. 2016). Provenances may also differ in their phenotypic plasticity in acclimation to drought, which is typically evident through leaf traits affecting gas exchange and plant water status (Robson et al. 2012; Sánchez-Gómez et al. 2013). The origin of different beech provenances can also affect seedling performance because of interactions with the microbiome of soil communities (Manzanedo et al. 2018), feeding back to effect drought tolerance. Besides location, the elevation of origin affects a suite of leaf-level traits related to stress tolerance and photosynthesis, as found in a comparison of five beech provenances along an elevational gradient in Czechia (Kučerová et al. 2018). Intraspecific differences in strategy involving trade-offs between growth and defense or stress tolerance are also visible in the metabolite profiles of leaves across beech provenances (Aranda et al. 2017). This variability can result from differences in the amount and composition of solar radiation received at their environment of origin, and even from differences in exposure to ultraviolet radiation (UV) (Neitzke and Therburg

2003). However, knowledge about the dynamics and drivers of secondary-compound accumulation in different beech population is sparse.

Our objective was to identify the processes underpinning the capacity of beech seedlings from populations of different origin to resist drought stress at high and low irradiances. To do this, we performed an experiment under semi-controlled conditions over one growing season, to test how imposed drought and sun-shade treatments affected plant traits indicative of water status, photoprotection and carbon assimilation in seedlings of *Fagus sylvatica*. We selected populations originating from four contrasting environments to grow under near-ambient temperature and radiation regimes in Helsinki, southern Finland. This also allowed us to test the extent of local adaptation in drought response across marginal and range-core populations. In general, marginal plant populations are expected to express greater stress tolerance, in a trade off against poorer performance in terms of maximum growth rate without stress (Rose et al. 2009; Bontemps et al. 2016). Accordingly, we tested the following hypotheses:

- We expected the provenance from the southern range-limit to perform best under water limitation because of its high physiological plasticity to acclimate to drought stress, in both sun and shade conditions (Hypothesis 1).
- From the core of beech distribution, we expected a faster growth rate and greater drought tolerance from the low-elevation provenance; but higher constitutive photoprotection, expressed as greater accumulation of UV-screening epidermal flavanols and hydroxycinnamic acids (HCA), in the provenance from high elevation (Hypothesis 2).
- The degree of stress tolerance might also reflect likelihood to migrate to higher elevations under climate change; a scenario sometimes predicted for beech populations (Sabaté et al. 2002). Low photosynthetic rates and stomatal conductances under stressful summer conditions, of high evaporative demand and low soil moisture, have previously been reported for northern range-edge provenances compared to the other beech populations (Aranda et al. 2012; Robson et al. 2012; Sánchez-Gómez et al. 2013). Hence, we expected the provenance from the northern range margin to have the lowest capacity to cope with drought stress during this experiment, reflected in lower photosynthetic rates in the sun and poorest control of water loss in general (Hypothesis 3).

Materials and methods

Plant material and experimental conditions

Four European beech (*Fagus sylvatica*) provenances from contrasting environments spanning a wide latitudinal gradient covering the species range were used in the experiment. Beechnuts were collected in autumn preceding the experiment from at least 20 trees per population and stored over winter in Helsinki. A relict population from the ancient beech forest of *Montejo de la Sierra* to the north of Madrid province (Montejo-ES) represented the south-westerly range margin of beech distribution. A population was chosen from *Blaviksliarna, Simlangsdalen,* in southern Sweden at the northern range margin of beech distribution (Blaviksliarna-SE). Two provenances were chosen from the core beech distribution in Germany at contrasting elevations: at *Eichelberg* (Eichelberg-DE, 525 m a.s.l.) and *Rindelpholz* (Rindelpholz-DE, 1175 m a.s.l) (Table 1).

All beechnuts were cleaned and fully imbibed in water (2nd February), and sown in seed trays of coarse sand, where they were kept moist and stratified at 3 °C for 8 weeks in a growth room with a photoperiod of 8-h light/16-h darkness. Germination of the four provenances was sequential (Table S1), so to synchronise the seedlings for the start of the experiment, later germinating cohorts were forced in a greenhouse at 20 °C, while early-germinating seedlings were held close to the two-cotyledon stage in cooler temperature conditions in an unheated greenhouse. Germinated seedlings were transplanted to individual tubes (20-cm length × 3.5-cm diameter) in a substrate of 3:1 sand:peat containing slow release fertiliser (Osmocote Exact Hi Start:15% N + 4.4% P_2O_5 + 8.3% K_2O + 1.8% MgO + TE, Scotts International B.V, Netherlands). During May, at the start of the experimental treatments all seedlings were transferred to an open-sided greenhouse with a roof blocking precipitation but transparent to 80% solar radiation (Table 2).

Table 1 Origin of the four beech provenances and climatic conditions at their origin

Provenance name	Latitude	Longitude	Elevation m a.s.l	Mean Annual Temperature	Mean Annual Precipitation
Montejo de la Sierra, Spain (Montejo-ES)	N 41 01′	W3 5′	1400 m	9.5 °C	1100 mm
Rindelpholz, Germany (Rindelpholz-DE)	N 47 29‴	E10 08″	1175 m	4.8 °C	2535 mm
Eichelberg, Germany (Eichelberg-DE)	N 48 55′	E11 26″	525 m	7.8 °C	692 mm
Blaviksliarna, Sweden (Blaviksliarna-SE)	N 57 90‴	E13 13′	75 m	6.5 °C	860 mm

Table 2 Midday clear (1st June) and cloudy sky (6th June) solar PAR irradiance (μmol m^{-2}) under each plot receiving shade and sun treatments

Treatment combination ($n=4$)	Cloudy day at midday mean ± SE	Sunny day at midday mean ± SE
Ambient outside	674 ± 37	1451 ± 74
Shade dry	284 ± 52	311 ± 50
Shade wet	237 ± 48	315 ± 77
Sun dry	594 ± 77	1222 ± 187
Sun wet	561 ± 72	1073 ± 203

Measured with a calibrated quantum sensor (LICOR 190). Each measurement is an average over 15-s intervals. Mean ± SE of four replicates of each treatment combination, within each replicate several measurements were made to capture the spatial variation in irradiance within a plot

In total 350 seedlings of similar height at the two-leaf stage were selected for use in the experiment and an equal number of seedlings from each provenance were allocated at random to each of eight "plots" (Fig. S1). The main plot factor was light treatment, and four replicate plots received near-ambient sunlight ("Sun"), while seedlings in four identical plots grew under a double-thickness of neutral shade cloth (Varjostuspeite K-Garden, Finland), where they received about 20–25% of ambient solar radiation ("Shade"). Light treatments were checked by measurements of photosynthetically active radiation (PAR) with a calibrated quantum sensor (LICOR 190, Li-Cor Inc., Lincoln, NE, USA): mean midday clear-sky solar spectral irradiance (μmol m^{-2}) under the treatments was 1147 μmol photons m^{-2} s^{-1} Sun and 313 μmol photons m^{-2} s^{-1} Shade on 1st June (Table 2). The watering treatment was a split-plot factor: half of the seedlings in each plot from both the Sun and Shade treatments were well watered ("Wet"), while the other half were subject to a water-limited treatment ("Dry") lasting from the start of the experiment on 24th May throughout the summer until mid-September. Soil moisture content was monitored both prior to and after watering throughout the experiment using a TDR probe (SM200 Moisture Sensor with HH2 Moisture Meter, Delta-T Devices, Cambridge, UK). This gave a % *v*/*v* soil moisture which could be expressed as soil water potential (Ψ_{soil}) using a calibration curve for the specific substrate mixture used in the experiment. A measured volume of water (usually 10–20 ml) was administered to each pot every 2–3 days to maintain soil moisture content at 10–15% *v*/*v* in the dry treatment, and 20–35% *v*/*v* in the Wet treatment (Fig. S2). The average temperature and relative humidity every 10 min throughout the experiment was monitored in each plot with an ibutton sensor (Maxim Integrated, San Jose, CA, USA; Fig. S3). The relative performance of seedlings from each provenance under each of the treatment combinations was compared through the summer by monitoring growth, leaf gas exchange, absorption by leaf pigments and leaf water potential.

Gas exchange measurements

The leaf-level photosynthetic rate and stomatal conductance were measured with a portable Infra-Red Gas Analyser (IRGA LICOR 6400; leaf chamber 2-×-3 cm; Li-Cor Inc., Lincoln, NE, USA). Measurements were taken on sunny days in late July (22nd, 23rd, 26th and 27th July) at mid-late morning (10:00–12:00) when photosynthetic rate was expected to be at its daily maximum. In total 340 measurements of light-saturated gas exchange were made from seedlings in each of the four replicate plots (up to 20 per provenance-treatment combination). Measured leaves received 1200 μmol photons m^{-2} s^{-1} PAR, provided by a LICOR6400-02B red-blue light source: this value was considered light-saturating as it exceeded the maximum light received by leaves at midday in the Sun and Shade treatments (Table 2). Set conditions in the leaf chamber were: flow rate 700 μmol s^{-1}, [CO_2] 380 ppm, *c*. 30% Relative Humidity (RH), equivalent to vapour pressure deficit (VPD) 2.4 kPa, and block temperature 22.1 °C, giving an air temperature of 23–24 °C. Measurements were recorded when the photosynthesis (A_{net}), stomatal conductance (g_s), and internal carbon dioxide (C_i) were stable. Additional parameters from the gas exchange measurements were calculated: the ratio of internal to ambient CO_2 concentration (C_i/C_a), transpiration rate (E_{wv}) and the instantaneous water use efficiency (IWUE A_{net}/g_s).

Leaf water potential measurements

The midday leaf water potential (Ψ_{midday}) was measured on the 3rd or 4th leaves produced by seedlings from each of the 4 replicate plots (in total *c*. 20 plants per provenance-treatment combination) on cloud-free days at midday on the same late July days as gas exchange measurements. These leaves had not been shaded by others and were excised at the petiole, recut with a scalpel, and their water potential immediately measured using a pressure chamber (model 1000; PMS Instrument Co., Albany, OR, USA). The specific soil-leaf hydraulic conductance (K_L) was estimated from water potential and gas exchange measurements as

$$K_L = E_{wv}/\left(\Psi_{soil} - \Psi_{midday}\right).$$

Soil water potential (Ψ_{soil}) measured, as described above, on the same morning as physiological measurements was used as a substitute for leaf pre-dawn water potential in the calculation of water potential difference ($\Psi_{diff} = \Psi_{soil} - \Psi_{midday}$), because the near-24-h daylength and small number of leaves per seedlings made it

impractical to measure pre-dawn leaf water potential. This relies on the assumption that pre-dawn soil water potential and pre-dawn leaf water potentials of beech seedlings are expected to be approximately in equilibrium (Sack and Holbrook 2006; Rose et al. 2009; Bolte et al. 2016), and thus the water potential di erence ($_{di}$) to $_{midday}$ can be interpreted as a coarse proxy for the leaf water potential adjustment between the daily minimum and maximum water loss.

Measurements of leaf and seedling growth and photoprotection

Growth measurements were made immediately prior to the experiment (23rd May) and on four occasions during the experiment (1st June, 28th June, 16th July, 19th August). Each set of measurements included seedling height, number of leaves, and number of branches of all seedlings in the experiment and leaf size (length and width) of the second leaf produced. Any variation in leaf colour, shape or injury was noted at the same time as these measurements. Leaf and seedling growth rates were calculated as the best t to a polynomial function. At the end of the experiment (19th August), measured leaves were sampled for calculation of leaf mass area (LMA) from the oven-dried mass of leaves and fresh leaf area of scanned leaves (Image-J, following Wang 2016).

An optical index was recorded of leaf adaxial epidermal avonols (Dualex 3.3 FLAV, Force-A, Paris, France—reference at 375 nm) three times during the experiment: Date 1 (June 1st), Date 2 (29th June and 3rd July), and Date 3 (14th July). At Date 1 and Date 3, an optical index of chlorophyll content (SPAD Konica Minolta 502 chlorophyll meter, Tokyo, Japan) was also recorded, and on Dates 2 and 3 an optical index of hydroxy-cinnamic acids (Dualex HCA, Force-A—reference at 315 nm) was recorded.

Statistical analysis

E ects of provenance and light conditions (Sun/Shade) considered as the main plot factors, and water stress (Dry/Wet) as a split-plot factor, were assessed using a mixed model ANOVA, where a pair of adjacent plots were considered a block (random factor: nested with light and water treatments). There were four replicate pairs of light treatments within the greenhouse, each containing both Wet and Dry